Potential Erroneous Assignment of Nonlinear Data to the Classical Linear Two-Compartment Open Model

ANTHONY R. DiSANTO* and JOHN G. WAGNER▲

Abstract □ The classical two-compartment open model implies that if the dose is progressively raised the amount of drug transferred to tissue is directly proportional to dose. A nonlinear model considered in this report assumes that the amount of drug transferred to tissue is a curvilinear function of dose due to a limited capacity of the tissue to bind drug. Simulated (C,t) data, generated from the nonlinear equation appropriate to the model, were found to be fit perfectly or almost perfectly to a double-exponential equation. The usual interpretation would lead to assignment of the classical two-compartment open model. The data reported emphasize the concept that with the limitations of assay sensitivity, several different doses of a drug need to be administered intravenously to elaborate the appropriate pharmacokinetic model.

Keyphrases □ Model, open, classical linear two compartment—equations relative to erroneous assignment of nonlinear data □ Pharmacokinetic models—erroneous assignment of nonlinear data to classical linear two-compartment open model, equations □ Drug transfer—linear two-compartment open model, erroneous assignment of nonlinear data

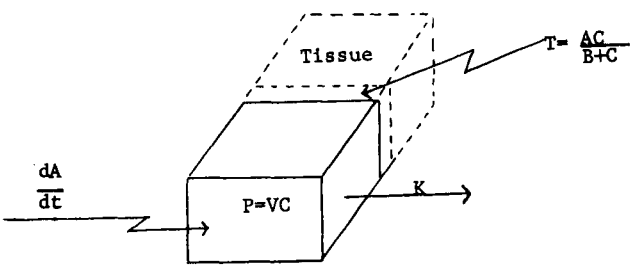
Deviations from linearity in pharmacokinetics may occur due to the absorption process, distribution, tissue binding and plasma protein binding of the drug, metabolic processes, and excretory processes. Krüger-Thiemer (1) summarized the majority of these deviations so they will not be presented here.

There is much experimental evidence that many drugs bind to tissues. The drug–tissue "reaction" may be one or more of chemical combination (*i.e.*, covalent bonding), simple electrostatic or van der Waals-type binding, complexation, adsorption of the drug on the surface of the cells, *etc.* It is also obvious that a limited amount of each tissue can bind a given drug and the amount of drug that can be taken up by a given tissue is related to some type of affinity constant. The classical two-compartment open model does not take this into consideration, since the model assumes distribution between the two compartments is controlled by two first-order rate constants. Hence, the classical model really implies that if the dose is progressively raised the amount of drug transferred to tissue is directly proportional to the dose.

There is also increasingly more evidence that following intravenous injection of many drugs the binding of the drug by the tissue is extremely rapid and the debinding, or release of drug from the bound state, is a much slower process. For example, Drach *et al.* (2) reported that within 1 min. after intravenous injection of diphenhydramine to the rhesus monkey, a large portion of the dose was bound to tissue, where it presumably was slowly metabolized and/or the metabolites of the drug were slowly returned to plasma. Within 3 min. after intravenous injection of indocyanine green, hepatic uptake of the dye in the rat was apparently near completion (3). Although the authors evaluated their data by relating the rate of uptake to the dose by the Michaelis–Menten equation, the same data may equally well have been explained by relating the amount of drug taken up by the liver to the dose. The nonlinear kinetics for kanamycin concentration in perilymph from the scala vestibuli of guinea pigs, reported by Stupp *et al.* (4), and the uptake of bromsulfalein by the reticulo–endothelial system, reported by Tovey (5), could also probably be explained in a similar manner. In studies with rats given 2-, 5-, 7.5-, 10-, and 25-mg./kg. doses of methylene blue by rapid intravenous injection, an average of 29.8% of the dose (range 25.2–35.8%) was recovered in only four tissues (heart, lung, liver, and kidney) when the animals were sacrificed 3 min. after injection (6).

A generalized nonlinear pharmacokinetic model was elaborated by DiSanto and Wagner and published by Wagner (7). This model takes into account the nonlinear tissue binding of drug to one or more tissues associated with each of two fluid compartments. The model incorporates concepts similar to those utilized by other investigators (1, 8–13), but many of the equations derived are distinctly different than those derived by these authors. Krüger-Thiemer *et al.* (11) discussed and illustrated curvature of a semilogarithmic plot of terminal total plasma concentration of a drug against time. The cause of the curvature in this case was assigned to strong plasma protein binding of the drug. They derived an equation, which is similar in form but not the same as Eq. 1 of this report, when symbols are redefined and plasma protein binding is involved rather than tissue binding. Many drugs are either loosely bound or not bound at all to plasma proteins but are highly tissue bound. For such drugs the semilogarithmic plot of terminal total plasma concentrations of drug *versus* time may also be curved in an analogous manner. However, the curvature, even after relatively "high" doses in a clinical sense, may not be observed due to limitations of assay sensitivity. It is this point we want to emphasize in this report.

Simulated data in this report show that in such cases "plasma concentrations" following several doses of



Scheme I—Heterogeneous one-compartment open model with binding to one type of tissue. For bolus intravenous injection, dA/dt = 0.

Table I—Data Simulated with the One-Compartment–One-Tissue Model According to Eq. 1, with C_0 Varied, and Fitted by NONLIN to the Classical Two-Compartment Open Model (Weighted Reciprocally)[a]

Variables		Estimated Parameters of Classical Model				Measures of Fit	
Dose[b], mg./kg.	C_0, mcg./ml.	k_{12}, hr.$^{-1}$	k_{el}, hr.$^{-1}$	k_{21}, hr.$^{-1}$	V_1, l./kg.	r^{2c}	Corr.[d]
29.90	50	0.450 (0.017)[e]	2.32 (0.018)	0.453 (0.020)	0.597 (0.003)	1.000	1.000
14.76	20	0.738 (0.034)	1.86 (0.023)	0.540 (0.028)	0.748 (0.007)	1.000	1.000
9.55	10	0.852 (0.048)	1.41 (0.024)	0.626 (0.038)	0.985 (0.013)	1.000	1.000
6.67	5	0.755 (0.044)	1.00 (0.016)	0.693 (0.041)	1.39 (0.016)	1.000	1.000
4.33	2	0.441 (0.024)	0.624 (0.006)	0.696 (0.034)	2.22 (0.016)	1.000	1.000
3.00	1	0.242 (0.010)	0.455 (0.002)	0.655 (0.023)	3.03 (0.011)	1.000	1.000
1.917	0.5	0.122 (0.004)	0.358 (0.001)	0.606 (0.017)	3.85 (0.006)	1.000	1.000

[a] Parameters of tissue binding model used to simulate data were A = 10.0, B = 1.0, K = 2.75, and V = 0.5; C_0 assigned: 50., 20., 10., 5., 2., 1., and 0.5. [b] Dose was calculated according to Eq. 2. [c] Coefficient of determination. [d] Correlation coefficient for regression of predicted and observed (*i.e.*, simulated) C values. [e] Standard deviation of the estimated parameter.

"drug" may be fit perfectly by the double-exponential equation appropriate to the classical two-compartment open model for bolus intravenous injection. It is also shown that if only one or two doses of such a drug were administered intravenously, one could erroneously conclude that this linear classical model applied. However, if five doses of the drug were administered, trends in the estimated parameters as a function of dose and inconstancy of the plasma clearance would indicate an inappropriate model. The fact that simulated data, generated from a simple nonlinear equation, may be fit perfectly by a double-exponential equation, when the parameters of the nonlinear equation are in a certain space, seems worthy of note. The data reported emphasize the concept that with the limitations of assay sensitivity, several different doses of a drug need to be administered intravenously to elaborate the appropriate pharmacokinetic model.

EXPERIMENTAL

When there is only one type of fluid and one type of tissue, the generalized model of DiSanto and Wagner (7) is greatly reduced (Scheme I) and leads to the nonlinear equation shown as Eq. 1 for the intravenous case:

$$t = \frac{1}{K}\left[\left(1 + \frac{A}{B}\right)\ln\left(\frac{C_0}{C}\right) + \frac{A}{B}\ln\left\{\frac{B + C}{B + C_0}\right\} + A\left\{\frac{C - C_0}{(B + C)(B + C_0)}\right\}\right] \quad \text{(Eq. 1)}$$

In Eq. 1, C represents the concentration of drug in the fluid volume (and is analogous to the plasma concentration of drug), C_0 represents the initial concentration at time zero immediately after the bolus dose, K represents the first-order rate constant for overall elimination of drug from the fluid volume with volume V, A represents the maximum amount of drug that can be taken up by tissue divided by the volume V, and B represents the dissociation constant of the tissue-bound drug divided by the volume V.

Sets of (C,t) data were generated using a digital computer program[1] based on Eq. 1 for the rapid intravenous case of the heterogeneous one-compartment open model with binding to one type of tissue (7). Seven sets of data were generated with the parameters A, B, K, and V held constant and C_0 varied. The dose was calculated with Eq. 2:

$$C_0 + \frac{AC_0}{B + C_0} = \frac{D}{V} \quad \text{when } t = 0 \quad \text{(Eq. 2)}$$

Sets of (C,t) data were also generated with the parameters K and C_0 held constant and A and B varied. All possible combinations were generated when A or B were assigned values of 0.003, 0.30, 3., 30., and 300. Since there were six values of A and six values of B, $6 \times 6 = 36$ sets of (C,t) values resulted.

Each of these sets, where applicable[2], was analyzed according to the two-compartment open model with rapid intravenous injection represented by Eqs. 3–5:

$$C_1 = \frac{D}{V_1(\alpha - \beta)}\left[(k_{21} - \beta)e^{-\beta t} - (k_{21} - \alpha)e^{-\alpha t}\right] \quad \text{(Eq. 3)}$$

where:

$$\alpha + \beta = k_{12} + k_{21} + k_{el} \quad \text{(Eq. 4)}$$

and:

$$\alpha\beta = k_{21}k_{el} \quad \text{(Eq. 5)}$$

Preliminary estimates of the parameters were obtained by the feathering or back-projection technique using semilogarithmic graph paper. Each applicable set of data was fitted by the method of least squares with an iterative digital computer program and an IBM 360/65 digital computer to conform to the appropriate equation (*i.e.*, Eq. 3). The graphical estimates of the parameters were used as starting values, and the concentrations were assigned reciprocal weights due to the large range of values. The least-squares estimates of parameters V_1, k_{12}, k_{el}, and k_{21} obtained by this procedure are listed in Tables I and II.

RESULTS

Simulated (C,t) data obtained from Eq. 1 over about a 3-cycle log range of C values were found to be readily fit by either a one- or two-term exponential equation (Tables I and II). In most of the parameter space of A, B, and K values, such simulated data were fit almost perfectly (r^2 value frequently equal to 1.00) by Eq. 3. The usual interpretation in such cases would lead to assignment of the

[1] The authors are grateful to Dr. C. M. Metzler, who wrote the DFUNC subroutine, for allowing use of his program NONLIN with nonlinear equations.

[2] Generated sets of (C,t) data which did not go over a 2- or 3-cycle log range were not used. This only occurred in the case where C_0 and K were held constant.

Table II—Data Simulated with the One-Compartment–One-Tissue Model According to Eq. 1, with C_0 Constant, and Fitted by NONLIN to the Classical Two-Compartment Open Model (Weighted Reciprocally)[a]

Variables		Estimated Parameters of Classical Model				Measures of Fit	
A, mg./kg.	B, mcg./ml.	k_{12}, hr.$^{-1}$	k_{el}, hr.$^{-1}$	k_{21}, hr.$^{-1}$	V_1, l./kg.	r^{2b}	Corr.[c]
0.3	0.003	0.482	2.58	0.300	0.993	1.000	1.000
		(0.014)[d]	(0.019)	(0.021)	(0.004)		
0.3	0.03	0.647	2.35	0.612	1.000	1.000	1.000
		(0.021)	(0.013)	(0.028)	(0.006)		
0.3	0.3	0.448	2.44	2.26	0.996	1.000	1.000
		(0.004)	(0.002)	(0.016)	(0.001)		
3.	0.03	1.37	0.993	0.482	1.047	0.999	1.000
		(0.073)	(0.032)	(0.044)	(0.019)		
3.	0.3	0.731	0.901	0.928	1.03	1.000	1.000
		(0.042)	(0.012)	(0.064)	(0.001)		
30.	3.	0.083	0.351	0.560	1.003	1.000	1.000
		(0.005)	(0.001)	(0.035)	(0.002)		
0.30	0.003	0.066	2.96	0.239	0.993	1.000	1.000
		(0.002)	(0.008)	(0.018)	(0.004)		

The following parameters of the tissue binding model resulted in a single exponential. Data were fit to classical one-compartment model by a least-squares line: $\ln C = \ln C_0 - Mt$, where M is the slope. The C_0 and K are as above.

A, mcg./ml.	B, mcg./ml.	Slope, hr.$^{-1}$	Intercept	Corr.[c]
0.03	0.3	2.792	0.951	1.000
0.03	3.	2.973	0.998	1.000
0.3	3.	2.742	0.986	1.000
0.3	30.	2.972	1.000	1.000
3.	3.	1.562	0.921	1.000
3.	300.	2.971	0.998	1.000
300.	3.	0.046	0.991	0.999
300.	30.	0.279	0.993	1.000

[a] Parameters of tissue binding model used to simulate data were: $C_0 = 1.0$ and $K = 3.0$. The parameters A and B were varied as indicated. [b] Coefficient of determination. [c] Correlation coefficient for regression of predicted and observed (*i.e.*, simulated) C values. [d] Standard deviation of the estimated parameter.

classical two-compartment open model from which k_{12}, k_{21}, k_{el}, and V_p would be calculated from the parameters of Eq. 3. Deviations from the fit of Eq. 3 to data generated with Eq. 1 occurred at very low C values, but in actual practice such low C values would usually not be capable of being observed due to limitations of assay sensitivity (Fig. 1). It would be very difficult, if not impossible, to make the correct model assignment on the basis of data collected after only one or two doses. However, when simulated (C,t) data were generated with Eq. 1 for seven different doses, and each set of (C,t) data was fitted according to Eq. 3, then the calculated values of k_{12}, k_{21}, k_{el}, and V_p showed systematic trends in relation to dose, even though the values of A, B, and K of Eq. 1 were held constant and only the C_0 varied (Table I). Also, in other such multiple simulations with constant C_0 and K values but variable A and B values, plots of the area $0 \rightarrow \infty$ *versus* dose, where the areas $0 \rightarrow \infty$ were estimated by integration of Eq. 3, were S-shaped. In some cases the plot was essentially linear but had a significant intercept.

Integration of Eq. 1 yields:

$$\int_0^\infty C \cdot dt = \int_0^{C_0} t \cdot dC = \frac{C_0}{K}\left(1 + \frac{A}{B + C_0}\right) = \frac{D}{VK} \quad \text{(Eq. 6)}$$

In each of the above cases, estimation of the area $0 \rightarrow \infty$ by the appropriate equation, Eq. 6, led to linear area $0 \rightarrow \infty$ *versus* dose plots that passed through the origin.

DISCUSSION

The simulations strongly suggest that to determine whether the classical two-compartment open model or a nonlinear model, such as the heterogeneous one-compartment open model with binding to one type of tissue, is the best explanation of real plasma or whole blood concentration–time data, one must measure such concentrations after several (preferably four to six different) doses of drug spread over a reasonable range. Also, the measurement of drug in several tissues of small animals as a function of dose, and shortly after intravenous injection, also aids in determining whether a nonlinear model or the classical model applies.

Common to all methods of estimating the extent of bioavailability after oral administration of a drug from plasma, serum, or whole blood concentration data is the necessity of estimating the area $0 \rightarrow \infty$ under the concentration curve. Usually, this area is estimated by Eq. 7:

$$\int_0^\infty C(t)dt = \int_0^T C(t)dt + \frac{C_T}{\beta} \quad \text{(Eq. 7)}$$

In Eq. 7 the integral on the right-hand side is usually estimated by means of the trapezoidal rule from observed concentrations; C_T represents the estimated concentration at time T, where T is the time that the log-linear phase commences; and β represents the rate constant calculated from data in the log-linear phase. Hence, the second term on the right-hand side of Eq. 7 is an estimate of the area under the curve from time T to ∞. If real data actually arise from nonlinear kinetics, then Eq. 7 would be inappropriate to estimate the total area. For the classical linear compartment models upon which Eq. 7 is based:

$$\left[\frac{d \ln C}{dt}\right]_{\substack{t \rightarrow \infty \\ c \rightarrow 0}} = -\beta \quad \text{(Eq. 8)}$$

However, for nonlinear models, Eq. 8 is invalid. For example, for the one-fluid–one-tissue model which leads to Eq. 1, it was shown (7) that:

$$\left[\frac{d \ln C}{dt}\right]_{\substack{t \rightarrow \infty \\ c \rightarrow 0}} = \frac{-K}{1 + A/B} \quad \text{(Eq. 9)}$$

Hence, these simulations and the discussed considerations have also emphasized problems involved in estimating the area under the concentration curve beyond the last sampling time. The limitations imposed by assay sensitivity and the mentioned considerations make this a real problem in bioavailability testing.

It should be emphasized, as Krüger-Thiemer (13) pointed out, that nonlinear models will provide linear area $0 \rightarrow \infty$ *versus* dose

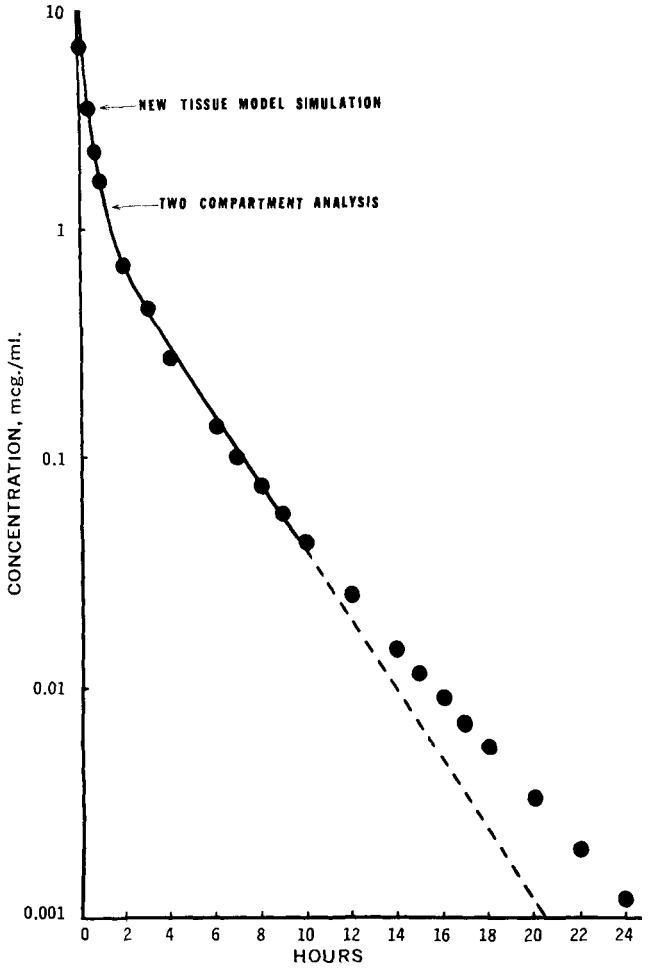


Figure 1—*Data simulated with Eq. 1 fitted to the two-compartment open model. The following values were used in this simulation:* C_o = *10 mcg./ml.*, B = *1.0 mcg./ml.*, A = *10.0 mcg./ml.*, K = *2.75 hr.*$^{-1}$, *and* V = *0.5 l./kg. Dotted line indicates extrapolation according to the two-compartment analysis. Concentration data were only generated for* t *up to 10 hr. After fitting the data to the classical model, concentration data were then generated up to 24 hr. to illustrate deviation.*

plots and that Eq. 10 of Wagner *et al.* (14) applies to many nonlinear models as well as linear models:

$$C_\infty = \frac{FD}{\overline{VK}\tau} \qquad \text{(Eq. 10)}$$

The principal requirement in such cases is that the drug must be lost from the central compartment by first-order kinetics. The result shown as Eq. 6 in this report is a specific example of a nonlinear case.

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* Present address: Clinical Bioavailability Unit, The Upjohn Co., Kalamazoo, MI 49001

▲ To whom inquiries should be directed.